

AD719548

FSTC-HT-23-964-70

US ARMY FOREIGN SCIENCE AND TECHNOLOGY CENTER

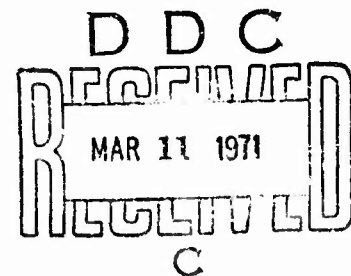


REPLICATION OF VEE VIRUS

by

V. M. Zhdanov, F. I. Yershov and L. V. Uryvaev

Country: USSR



*This document is a rendition of the
original foreign text without any
analytical or editorial comment.*

Distribution of this document is unlimited. It may be released to the Clearinghouse, Department of Commerce, for sale to the general public.

Reproduced by
NATIONAL TECHNICAL
INFORMATION SERVICE
Springfield, Va. 22151

9

TECHNICAL TRANSLATION

FSTC-HT-23-964-70

ENGLISH TITLE: REPLICATION OF VEE VIRUS

FOREIGN TITLE: PENLIKATIVIYOY KOMPLEKS VIRUSA VENESUZLYSKOGO
ZITSEFALOMIYELITA LOSHADEOY

AUTHOR: V. M. Zhdanov, F. I. Yershov and L. V. Uryvaev

SOURCE: DOKLADY AKADEMII NAUK SSR, Vol. 189, No. 6, 1969,
pp. 1382-1384

Translated for FSTC by Techtran Corporation

NOTICE

The contents of this publication have been translated as presented in the original text. No attempt has been made to verify the accuracy of any statement contained herein. This translation is published with a minimum of copy editing and graphics preparation in order to expedite the dissemination of information. Requests for additional copies of this document should be addressed to the Defense Documentation Center, Cameron Station, Alexandria, Virginia, ATTN: TSR-1.

This translation was accomplished from a xerox manuscript. The graphics were not reproducible. An attempt to obtain the original graphics yielded negative results. Thus, this document was published as is, in order to make it available on a timely basis.

It has been demonstrated earlier [1] that in the cells of the fibroblasts of a chicken embryo which have been infected with Venezuelan Equine Encephalitis virus a double-helix replicative form (RF) RNK develops, becoming converted into a multiple-helix replicative intermediary form (RPF), which ensures the synthesis of daughter RNA and some forms of functional RNA with sedimentation constants from 19 to 38 S. On the model of RNA-containing phages it was determined [2] that the multiple-helix replicative intermediary form associates itself with the fibosomes of the host cells, forming a so-called replicative complex (RK), which ensures simultaneously replication and the synthesis of virus-specific proteins.

In the present communication we show that the replicative intermediary form of VEE functions in cells infected with the virus in the replicative complex form.

The VEE - chicken fibroblasts system described earlier [3] was used. The infecting dose of the virus came to 50-1000 bo. units per cell, actinomycin D (2 μ g/ml) were added immediately after contact of the virus with the cells, the newly synthesized virus RNA was labelled with H^3 -uridine (2-5 μ C/ml), and the proteins with C^{14} -aminoacid hydrolyzate of chlorella (2 μ C/ml) at various periods after infection.

Destruction of the cells was carried out in a homogenate buffer (tris HCL 0.05 M pH 7.4; KCl 0.05 M; $MgCl_2$ 0.005 M; saccharose 0.25 M) with the help of a Downs homogenizator. The nuclei and the residue of cytoplasm were precipitated by centrifuging at 3,000 g, the so-called mitochondrial fraction at 15,000 g, and the light polysomes and monosomes were separated from the supernatant at 100,000 g. RNA was separated out by the cold detergent-phenol method [4] from all fractions; in addition, RNA was separated out from the nuclei at 70° [5]. Analysis of the virus-specific polysomes was carried out after centrifuging in a saccharose gradient of 17-40 and 15-60%. The radioactivity count was carried out in a Packard fluid scintillation counter after precipitation of the material with trichloroacetic acid in milliporous filters.

In Figure 1 we show the distribution of the labels of the newly synthesized RNA by cell fractions from 3 to 4 hours after infection. As is apparent from these data, the main quantity of the newly synthesized RNA is contained in the nuclei and the mitochondrial fraction, in which connection double-helix RNA (RF) predominates in the nuclei, while in the fraction of the mitochondriae and the heavy polysomes single-helix RNA (replicative intermediary form) predominates. In the infected cells processed with actinomycin D the inclusion of H^3 -uridine was insignificant, RNA resistant to ribonuclease as detected in the double-helix form, also in insignificant quantities (transport RNA).

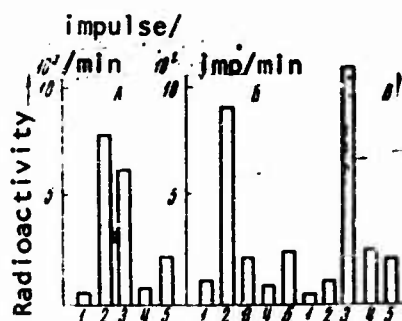


Figure 1. Distribution of Stain (H^3 -Uridine) of Newly Synthesized RNA in Fractions of Cells of Chicken Fibroblasts Infected with VEE. H^3 -uridine was added 3 to 4 hours After Infection. A. Total RNA; B. Supernatant after processing 2 M NaCl; C. Precipitate after processing 2 M NaCl. Tests B and C were processed with ribonuclease (15 μ g/ml). 1. RNA from fragments of cells, extracted at 4° ; 2. RNA from nuclei, extracted at 70° ; 3. RNA from fractions of mitochondriae and heavy polysomes; 4. RNA from fraction of polysomes; 5. RNA from supernatant after precipitation of polysomes.

Upon centrifuging of the cell homogenate in the 17-40% saccharose density gradient after precipitation of the mitochondrial fraction, the label of the newly synthesized RNA of the protein was detected in the polysomes, to begin with in the 160-180 S field, then in later time periods in the 250 S field and in the precipitate (Figure 2). Inasmuch as with this system the heavy polysomes settle on the bottom of the test tube, tests were set up with centrifuging of the homogenate of infected cells in the 15-60% saccharose gradient without preliminary precipitation of the mitochondrial fraction at 15,000 g. In Figure 3 we show the results of one of the tests. As is apparent from these data, the basic label of the newly synthesized RNA and proteins settles in the fraction of the heavy polysomes (the so-called mitochondrial fraction).

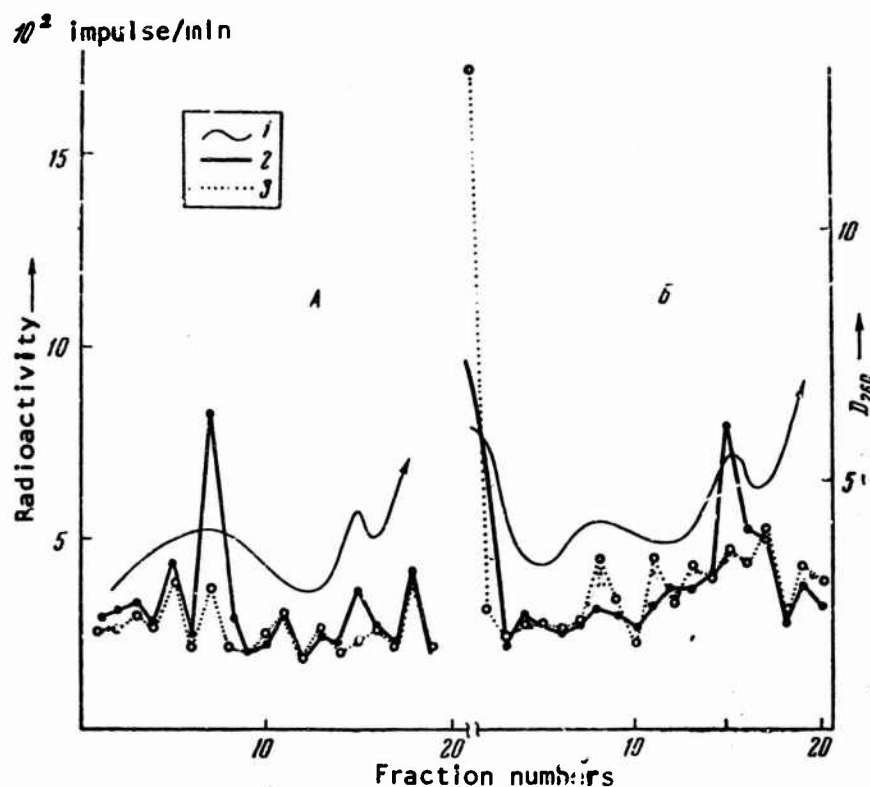


Figure 2. Distribution of Stain of Newly Synthesized RNA (H^3 -uridine) and of Protein (C^{14} -aminoacid mixture) upon Polysomes of cells of Chicken Fibroblasts Infected with VEE. A. Isotopes added 1 to 1 1/4 hours after infection; B. 2 1/2 to 2 3/4 hours after infection. Centrifuged in 17-40% Saccharose gradient at 25,000 rpm for 1 1/2 hours. 1. Optical density; 2. Inclusion of H^3 ; 3- C^{14} . Bottom of gradient to left, top to right.

Since the basic part of the multiple-helix virus RNA (replicative intermediary form) is found in this same fraction, it seemed logical to study its sedimentation properties. In Figure 4 we set for the results of one of the texts. As is apparent from these data, ribonuclease-resistant RNA separated out from the heavy polysomes is heterogeneous and has constants of sedimentation from 18 to 30 S with a principal peak in the range 25-26 S, i.e., it corresponds completely to the replicative intermediary form of this virus earlier described by us.

Inasmuch as it is associated with ribosomes one may draw the conclusion that the replicative intermediary form in the infected cells functions

in the form of a replicative complex upon which the replication of the virus RNA and the synthesis of the virus-specific proteins run their course simultaneously.

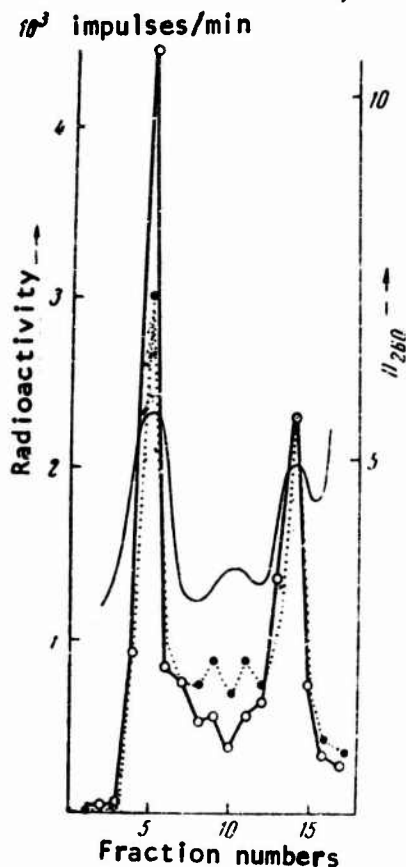


Figure 3. Distribution of Newly Synthesized RNA and of Protein on Polysomes of Cells of Chicken Fibroblasts Infected with VEE. Centrifuged in Saccharose Gradient 15-16% at 25,000 rpm for 2 1/2 hours. Designations same as in Figure 2.

D. I. Ivanovski Institute of Virology
Academy of Medical Sciences of the USSR,
Moscow

Received by editors on February 19, 1969.

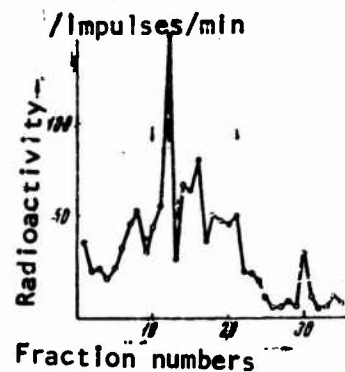


Figure 4. Distribution of Stain of Newly Synthesized RNA of VEE, drawn off from the field of the heavy polysomes and precipitated by 2 M NaCl, in the 5-20% saccharose gradient. Centrifuged at 20,000 rpm for 16 hours, fractions of gradient processed with ribonuclease (2 μ g/ml) after centrifuging. Positions of markers - RNA cell peaks at 28 S and 18 S respectively - are indicated by arrows.

References

1. Zhdanov, V. M., F. I. Yershov, L. V. Uryvaev, *Voprosy Virologii*, [Problems of Virusology], No. 3, 1969.
2. Hotham-Iglewski, B., R. M. Franklin, "Proceedings of the National Academy of Sciences of the U. S. A.," Vol. 58, p. 743, 1967.
3. Yershov, F. I., V. M. Zhdanov, *Acta Virologica*, Vol. 12, p. 136, 1966.
4. Zhdanov, V. M., *Voprosy Virologii*, [Problems of Virusology], No. 6, p. 686, 1968.
5. Georgiev, G. P., V. L. Mantseva, *Biokhimiya*, Vol. 27, p. 949, 1962.

UNCLASSIFIED

Security Classification

DOCUMENT CONTROL DATA - R & D		
(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)		
1. ORIGINATING ACTIVITY (Corporate author) Foreign Science and Technology Center US Army Materiel Command Department of the Army		2a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED
		2b. GROUP
3. REPORT TITLE REPLICATION OF VEE VIRUS		
4. DESCRIPTIVE NOTES (Type of report and inclusive dates) Translation		
5. AUTHOR(S) (First name, middle initial, last name) V. M. Zhdanov, F. I. Yershov and L. V. Uryvaev		
6. REPORT DATE 30 October 1970	7a. TOTAL NO. OF PAGES 5	7b. NO. OF REFS N/A
8a. CONTRACT OR GRANT NO. A. PROJECT NO. C. T702301 2301 d. Requester: Miss Perkins, AB	8b. ORIGINATOR'S REPORT NUMBER(S) FSTC-HT-23-964-70 9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report) ACSI Control Number (None)	
10. DISTRIBUTION STATEMENT This document has been approved for public release and sale; its distribution is unlimited.		
11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY US Army Foreign Science and Technology Center	
13. ABSTRACT The authors report the results of tests which they consider to demonstrate that in cells infected with Venezuelan Equine Encephalitis virus the replicative intermediary form of this virus functions in what they call a replicative-complex form, which ensures simultaneously both replication and the synthesis of virus-specific proteins.		

DD FORM 1473

1 NOV 65

REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

UNCLASSIFIED

Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Fibroblasts Polysomes Encephalitis virus Replicative complex Cytoplasm						